The Riddle of the Sphinx: Population Ecology of the Endangered Blackburn‘s Sphinx Moth, *Manduca blackburni* (Lepidoptera: Sphingidae) on an Invasive Host Plant.

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DEDICATION

This thesis is dedicated to my family: my two beautiful boys, Jacob and Phineas; my loving and supportive husband, Tim; and my Spartan mother, Barbara. Without your encouragement and support this could never have happened. I love you, I love you, I just love you!

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ABSTRACT

Blackburn‘s sphinx moth, *Manduca blackburni* (Lepidoptera: Sphingidae; Butler 1880), is a large sphinx moth endemic to the Hawaiian Islands. In 2000, it became the second moth and the first Hawaiian insect to be federally listed as an endangered species, yet little was known about its population biology. Its primary host plant is the invasive weed tree tobacco, *Nicotiana glauca* (Solanaceae). Despite the presence of tree tobacco on six of the seven main Hawaiian Islands, Blackburn’s sphinx moth is currently known from only Maui, Kaho‘olawe, and Hawai‘i Island where it occurs at densities well below what its invasive host plant would seem to support. In order to facilitate conservation efforts, I set out to analyze changes and patterns in abundance, elucidate ovipositional preferences, and identify the factors responsible for the apparent population suppression of this charismatic Hawaiian moth all on its novel invasive host plant.

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INTRODUCTION

Insect conservation faces numerous challenges including under-estimation of threat levels and extinction risks (McKinney 1999, Leandro et al. 2017) and insects are neglected in conservation research and funding relative to many other taxonomic groups (Clark and May 2002, Regnier et al. 2015). These biases have been attributed, in part, to a lack of baseline information on many insect species (Medeiros et al. 2013). Compared to vertebrate species that are typically perceived as charismatic, insects are often small, cryptic, and have naturally fluctuating population sizes, making many species difficult to observe for those lacking entomological training. Careful monitoring of at-risk insect species is needed to understand their population status as well as to identify and mitigate the threats they face. Yet, even for the more broadly appreciated insects such as butterflies and moths, it can be difficult to accurately assess abundance of endangered or declining species (Lindzey and Connor 2010).

Monitoring insect populations for conservation purposes primarily relies on counts of adults to detect changes in populations (Roy et al. 2007, Carlile et al. 2009, Longcore et al. 2010, Matteson et al. 2012, Roets et al. 2013, McKenna-Foster et al 2016, Putenney and Schorr 2016, Harvey et al. 2017). Adults are typically the most conspicuous and charismatic life stage and therefore garner the most research interest (Pollard and Yates 1993, Asher et al. 2001, Harker and Shreeve 2008, Van Sway et al. 2008, Pellet et al. 2012). However, many species violate the requirement of uniform and stable detection probabilities needed by more advanced models to explicitly use raw counts to estimate even relative abundance (Knutson et al. 1999, Gross et al. 2007, Collier et al. 2008). Mark-release-recapture (MRR) techniques are commonly used for estimating detection probabilities. Yet, MRR techniques call for repeated capture and handling of individuals which can be impractical, injurious, or even fatal for some fragile species (Murphy 1987) and can prove inaccurate for small populations (Knutson et al 1999). When working with endangered species, small populations are a common challenge. It is, therefore, difficult to justify such risks or receive the necessary

permitting to implement such a sampling scheme for endangered species most in need of monitoring.

Conversely, monitoring for pestiferous insect species successfully exploits detection across all life stages to track population changes (Beyo et al. 2004, Castle and Naranjo 2008, Mankin et al. 2010, Rogers et al. 2015). With a few exceptions (Thomas and Simcox 1982, Murphy and Weiss 1988, Longcore et al. 2010, Linzey and Connor 2010, Smee et al. 2011, Armstrong and Louw 2013), monitoring for conservation purposes largely ignores immature stages as a source of data on insect abundance, despite copious evidence that they can be statistically useful and even preferable for understanding population status across taxa (Sorenson et al. 1995, Novotny and Turcani 1997, Cameron et al. 2001, Hammack et al. 2003, Silva et al. 2019). Linzey and Connor, 2010, calculated detection probabilities and assessed the correspondence between abundance of immature and adult stages of the US federally listed endangered mission blue butterfly, *Icaricia icarioides missionensis* Hovanitz. They found that larvae and feeding damage had the highest detection probabilities and surveys of immature stages produced highly repeatable counts. This provides a method of estimating abundance for some species whose biology or conservation status make traditional survey methods impractical or impossible. It also indicates that species with immature stages that are easy to detect, count, and identify, will likely conform to the assumptions of uniform and stable detection probabilities needed to use raw counts to reliably estimate relative abundance.

Blackburn's sphinx moth, *Manduca blackburni* (Butler 1880) (Lepidoptera: Sphingidae), is a United States federally listed endangered species endemic to the Hawaiian Islands (USFWS 2005). The range of *M. blackburni* once included dry forest habitats on all of the main islands, except Kahoʻolawe. Three federally listed endangered species of the endemic genus *Nothocestrum* (Solanaceae) are the native larval host plant for Blackburn's sphinx moth. However, *M. blackburni* is oligophagous within the Solanaceae and quickly expanded onto the nonnative, invasive weed tree tobacco, *Nicotiana glauca* R. Graham, (Solanaceae).Following introduction in the late 1800s, tree tobacco quickly spread to six of the seven main Hawaiian islands. By 1919 it had become the most commonly used larval food plant for caterpillars of *M. blackburni* (Swezey 1924, Riotte 1986, Rubinoff and San Jose 2010). Despite this host range expansion, Blackburn's sphinx moth populations continued to decline for decades. After

extensive surveys in the 1970's failed to find the moth researchers believed it to be extinct, possibly due to non-native arthropod predators or parasitoids (Gagne and Howarth 1982). A single population was rediscovered on Maui in 1984, followed more than a decade later by rediscovery of populations on Kahoʻolawe and Hawaiʻi Island. Yet, *M. blackburni* populations only persist on three islands (USFWS 2005) at density levels well below what the invasive host plant could support (Rubinoff and San Jose 2010).

Conservation of species, especially insect species, requires understanding population status. Quantifying changes in abundance can be an essential tool for tracking changes in populations and requires an accurate and efficient methodology. This study was undertaken to assess whether surveys of immature stages provide an effective tool for understanding changes in relative abundance. The federally listed endangered Blackburn's sphinx moth, *Manduca blackburni* (Butler 1880) (Lepidoptera: Sphingidae), is a strong model as adults are nocturnal, occur at low-density, are highly vagile, and are rarely seen, even at light traps, necessitating an alternative method of surveying. The primary larval host plant, tree tobacco, is highly apparent in the landscape and immature stages of Blackburn's sphinx moth are easily identifiable. Immature stages are consequently likely to conform to stable detection probabilities.

METHODS

Study Sites

The study site selection was influenced by surveys conducted in 2011 and 2012 based at Puʻu Waʻawaʻa and Puʻu Anahulu in the North Kona district of Hawaiʻi Island. Those surveys were undertaken by researchers from the Hawaiʻi Department of Land and Natural Resources (DLNR) to estimate expected mortality of *M. blackburni* during clearing of the invasive weed, *N. glauca*, and included counts of all immature stages of *M. blackburni* and extensive mapping of the invasive host plant. Both locations are characterized by highly degraded, dry- forest habitat consisting primarily of the invasive plants *Pennisetum setaceum* (Poaceae), *N. glauca*, and *Lantana camara* (Verbenaceae). Scattered individuals of the native plants *Metrosideros polymorpha* (Myrtaceae), *Diospyros sandwicensis* (Ebonaceae), *Erythrina sandwicensis* (Fabaceae), *Santalum* sp. (Santalaceae), *Sida fallax* (Malvaceae), and *Dodonaea viscosa* (Sapindaceae) also occur within the landscape. The native host plant for *M. blackburni* on

Hawai‘i Island, *Nothocestrum breviflorum*, was never encountered during surveys even though it occurs in the region.

Survey Methods

Surveys utilizing similar methodology in the same regions were conducted in 2011 and 2012, making it possible to evaluate changes in abundance across a broader time scale. DLNR researchers surveyed 37 and 94 transects respectively in 2011 and 2012. I conducted all surveys from March 2017 to April 2019 with the help of volunteers. For all surveys in 2018 and 2019 all volunteer teams were under my direct supervision in the field to homogenize methodology and data collection.

Riotte (1986) described two flight periods for *M. blackburni*: January through April and June through October. However, surveys conducted by DLNR researchers at Pu‘u Wa‘awa‘a and Pu‘u Anahulu in August 2012 (unpublished data) found virtually no eggs or larvae present, indicating a difference in flight period for populations within the survey regions. At the survey sites, the presence of eggs and larvae seems to be closely associated with rainfall events (unpublished data) which are more common in October through April in the North Kona district of Hawai‘i Island. This informed our choice of survey dates. Volunteers were trained to identify and search *N. glauca* and confidently recognize all life stages of *M. blackburni*.

Belt transects (25 m x 3 m) were used by all research teams to monitor *M. blackburni* populations. In 2017 – 2018, survey teams created transects at randomly selected points from the known distribution (N = 979 GPS points) of the primary larval host plant. We oriented one 75 m^2 transect parallel to the firebreaks used to access the remote sites. Once the first transect was positioned, a second perpendicular 75 m^2 transect was placed at a random point adjacent to the first transect, ensuring non-overlapping transects, and including at least one host plant (Fig. 1.1). At some sites, due to low host plant density, it was only possible to establish either a parallel or a perpendicular transect but not both. Since firebreaks are manually and chemically cleared of all vegetation as needed for fire safety, transects never ran in, through, or across them. The exact number of transects and the number of times they were surveyed each year is listed in Table 1.1. Surveys occurred over 5-10 days and all surveys were conducted during one of the active reproductive seasons of the moth. The single yearly surveys in 2011, 2012, and 2017 typically occurred in February or March and each inspected a different set of randomly selected transects.

The monthly surveys in 2018 and 2019 are recorded in Figure 1.3 and utilized the same transects. We added nine randomly selected transects to those surveyed at Puʻu Waʻawaʻa in March of 2018 due to low abundance of eggs and larvae of *M. blackburni*. Those additional transects were included in all subsequent surveys. Also, at the start of surveys in 2019 I had to relocate three transects due to total loss of host plants from natural and anthropogenic causes. The replacement transects were located as close to the original transects as possible to maintain continuity. Field crews searched all host plants with trunks originating within the transects for immature stages of *M. blackburni*. We also recorded any other invertebrates seen on the host plants. The status of eggs was recorded as 1) predated if they were hollow, with a clear chorion and a hole too small to provide egress for a neonate larva; (2) parasitized by *Trichogramma* (Hymenoptera: Trichogrammatidae) if they were opaque pearlescent black (Flanders 1937, Kuhar et al. 2002); and (3) potentially viable if the eggs appeared developmentally normal. The instar (1-5) and color morph (green or purple) of larvae were recorded. We estimated plant height and leaf density as described in Chapter 2. Due to extreme host plant height or brittleness, some plants were impossible to search exhaustively. In those cases, we estimated the percentage of the plant fully searched. Mean percent searched ranged from 89.3% ± 1.30 to 97.2% ± 0.24 across all survey years (Table 1.1).

Figure 1.1 Example of survey site showing parallel and perpendicular transects.

Table 1.1 Transects surveyed by date and the average search coverage for host plants within transects

Year	Transects Surveyed	Times Surveyed	% Searched ± SEM
2011	37	1	95.01 ± 0.44
2012	94	1	97.16 ± 0.24
2017	164	1	89.98 ± 0.75
2018	87-96	2–3	92.93 ± 0.51
2019	96	3	89.39 ± 1.30

Statistical Analysis

I conducted all analyses using JMP Pro version 14.2.0. The mean number of *M. blackburni* immatures counted per transect was used as a proxy for relative abundance. Given the unequal variance across survey years in the data, pairwise nonparametric Wilcoxon tests were used to test for statistical significance of differences in relative abundance of *M. blackburni* and the invasive host plant *N. glauca* across all five survey years. Generalized linear regression fitted to a negative binomial distribution was used to determine significance of relationship between host plant abundance and moth abundance. Nominal logistic regression was used to determine significance of relationship between host plant abundance and moth presence.

Results

The estimated relative abundance of *M. blackburni* was statistically significantly higher in 2011 than all other years ($p \leq 0.01$). 2019 had the lowest relative abundance and was significantly lower than any other year ($p \leq 0.004$). There were no significant differences among relative abundances in 2012, 2017, and 2018 ($p \geq 0.21$) (Fig. 1.2). Extreme fluctuations in abundance were found in monthly surveys in 2018 and 2019 (Fig 1.3). While there was no significant difference in host plant abundance between 2011 and 2012, the relative abundance of *N. glauca* within transects decreased significantly ($p \leq 0.01$) each survey year after 2012 (Fig 1.4). Generalized linear regression analysis showed a significant correlation between the number of trees per transect and the abundance of *M. blackburni* ($\chi_2 = 4.4178$, $p = 0.0356$), and a highly significant relationship between the number of host plants per transect and the presence of *M.*

blackburni (χ_2 = 12.09, p = 0.0005). I also recorded the relative proportion of eggs found parasitized and predated in 2017, 2018, and 2019 (Table 1.2). In 2017, 58.2% of eggs had been parasitized or predated. In 2018 and 2019, 48.8% and 64.4% of eggs, combined across monthly sampling dates, had been predated or parasitized.

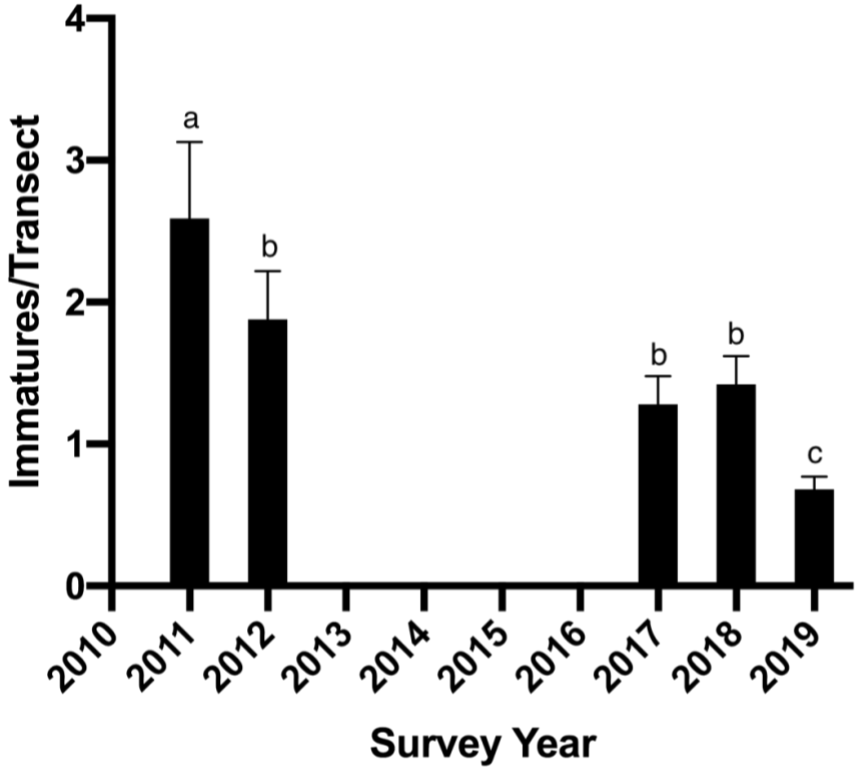

Figure 1.2 Relative abundance of *M. blackburni* calculated as the mean number of immatures per transect by survey year. Columns which do not share letters are significantly different at $p \leq 0.05$.

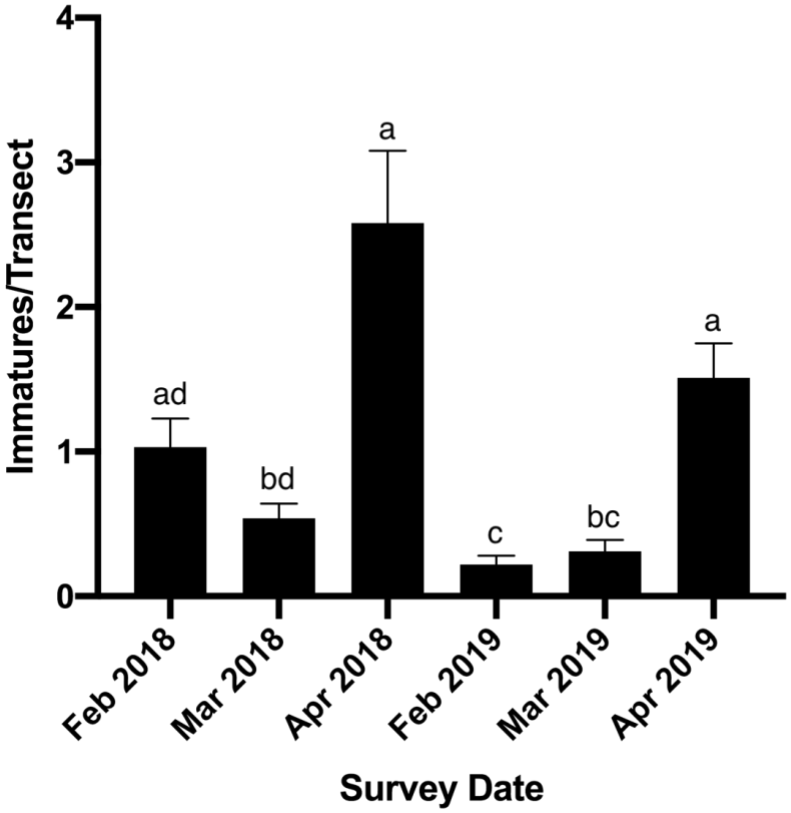

Figure 1.3 Relative abundance of *M. blackburni* displaying extensive intra-seasonal variation. Columns which do not share letters are statistically significantly different at $p \leq 0.05$.

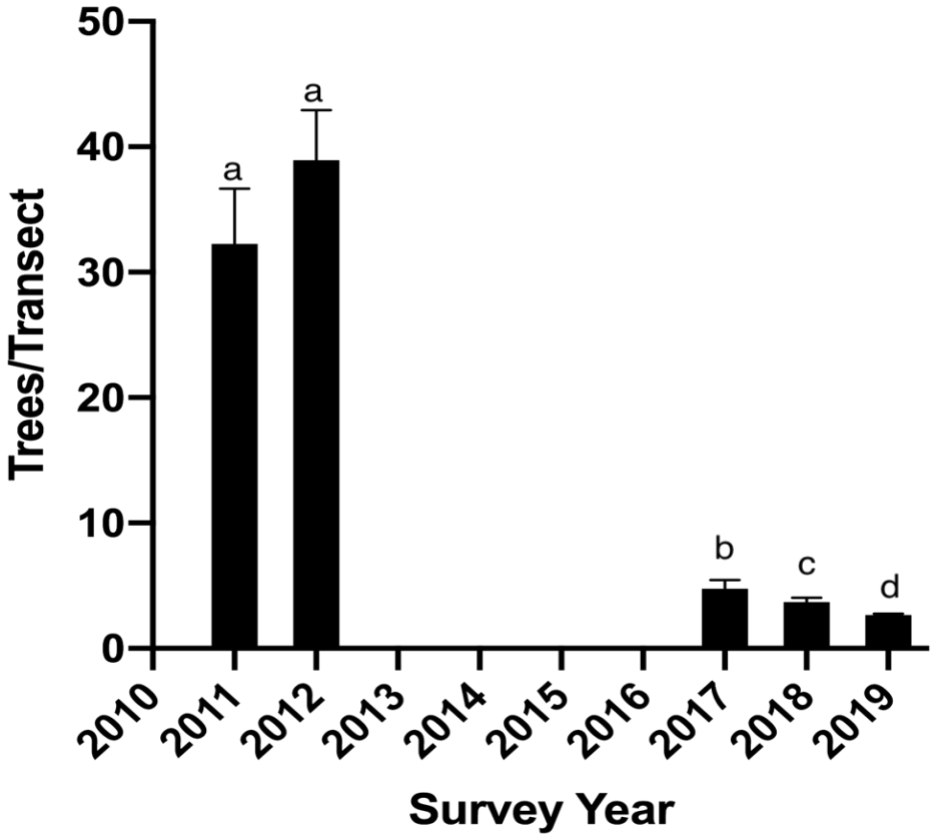

Figure 1.4. Host plant abundance calculated as the mean number of *N. glauca* plants per transect by survey year. Columns which do not share letters are statistically significantly different at $p \leq 0.05$.

Table 1.2 Counts of potentially viable and non-viable *M. blackburni* eggs by survey year indicating apparent egg mortality.

Date	Viable eggs	Non-viable eggs	Total eggs	Apparent egg mortality
2017	79	110	189	58.2%
2018	150	143	293	48.8%
2019	62	112	174	64.4%

* Egg counts from monthly surveys were combined by year
** Eggs which were found predated or parasitized were considered non-viable.

DISCUSSION

In 2011, the first randomized surveys for immature stages of *M. blackburni* were conducted by DLNR to estimate the number of eggs and larvae which would be lost when infestations of *N. glauca* choking firebreaks were removed. In the North Kona district, both natural and anthropogenic fires pose a significant threat to private property and conservation areas. Firebreaks are maintained at Puʻu Waʻawaʻa and Puʻu Anahulu to help protect native outplantings, prevent the spread of fires, and provide access to emergency crews. Clearing work did not influence the abundance of host plant within transects between 2011 and 2012. Transects were never placed within firebreaks during any survey years, to avoid bias introduced by doing so. Survey data from 2012 shows a statistically significant decline in relative abundance of *M. blackburni* from levels estimated in 2011. Removal of *N. glauca* from firebreaks in 2011 may have reduced general abundance of the host plant enough to produce the statistically significant reduction in relative abundance of *M. blackburni*. The current land management practice for clearing infestations of *N. glauca* in firebreaks recommends conducting removal during the dry season, when pupae are believed to aestivate (Tessler 2015), but also allows for clearing of smaller plants more frequently during the moth's active season. Rubinoff and San Jose (2010) found that ¼ of the larvae they reared built shallow pupal chambers less than 25 mm below the soil surface and directly under the rootball of their host plants. Therefore, clearing activities themselves, which include manual removal, all-terrain vehicles equipped with tow-behind mowers, tractors, and herbicides may have decreased survivorship of some pupating individuals through inadvertent destruction of pupae if pupal chambers were disturbed.

There is no way to know what fluctuations in relative abundance may have occurred during the four-year lapse in surveys spanning 2013-2016. However, the relative abundance of *M. blackburni* found by DLNR in 2012 was consistent with the relative abundance I estimated in 2017 and 2018. It is important to point out that survey teams found high apparent mortality through predation of eggs in 2017, 2018, and 2019 (Table 1.2). Recording apparent mortality of eggs was not a routine part of surveys performed in 2011 and 2012 so it is not possible to compare apparent mortality of eggs across all surveys. Also, while the relative abundance of *M. blackburni* did not change significantly, the relative abundance of the host plant exhibited a highly significant reduction in 2017 and continued significant declines in 2018 and 2019. During 2019, lowest host plant abundance was observed as well as lowest relative abundance of *M. blackburni*. Many host plants at Puʻu Waʻawaʻa displayed significant herbicide damage following efforts to clear *N. glauca* from the firebreaks. (Fig. 1.5). Plants that had come in contact with herbicide had lost virtually all adult leaves and exhibited crowded, stunted leaf growth with abbreviated petioles. The significant correlation between with-in transect tree tobacco abundance and both presence and abundance of Blackburn's sphinx moth helps explain the decreases in moth abundance over time. Both high levels of egg predation and significant declines in host plant abundance are likely to have influenced the significant decline in relative abundance of *M. blackburni* measured in 2019.

Management Recommendations

The significant decline in host plant abundance within the survey areas represents a concerning loss of resources for *M. blackburni,* especially in light of the correlation between host plant abundance and moth abundance and presence. A large portion of both survey regions used in this study are covered in the invasive African bunchgrass fountain grass, *Pennisetum setaceum,*(Forssk.) Choiv. (Poeaceae). Fountain grass is known to form dense monocultures which prevent colonization of other plant species (Adkins et al. 2011) and seems to be able to outcompete tree tobacco. In the two survey regions, I observed that seedling recruitment of *N. glauca* was limited to disturbed areas which had not been colonized by fountain grass, such as firebreaks. Since firebreaks are annually cleared of vegetation, both to prevent the spread of wild fires and to allow emergency vehicles access to fight fires when they occur, tree tobacco numbers are diminishing. Introducing disturbance outside of firebreaks would help maintain

Figure 1.5 Healthy *Nicotiana glauca* on the left and *N. glauca* with miniscule leaves and abbreviated petioles characteristic of herbicide damage on the right

larger tree tobacco populations without compromising fire safety. More careful application of liquid herbicides or use of a granular herbicide would help prevent damage to *N. glauca* in areas adjacent to firebreaks, thereby preserving reproductive and larval resources for Blackburn's sphinx moth. The use of grass-specific herbicides could clear areas overgrown by fountain grass, facilitating *N. glauca* seedling recruitment. Unlike fire adapted fountain grass, Hawaiian native forest plants are believed to have evolved without regular exposure to fire (Vogl 1975). As a result, many native plants are eliminated by fire and native forests are less capable of regeneration after fire than fountain grass (Adkins et al. 2011, Blackmore and Vitousek 2000). Therefore, fire creates a negative feedback loop which reduces native forest cover and increases the spread of invasive fire adapted grasses which in turns increases the risk of fire. Removing fountain grass would thus reduce fuel loads and abate the risk of wildfires. However, since pupation of *M. blackburni* occurs underground, there is a chance that pre-pupae and pupae could

come into prolonged contact with herbicides in the soil. Prior to application, it would be essential to ensure the herbicides used are not deleterious to *M. blackburni*.

While there has been a statistically significant decline in relative abundance of *M. blackburni* over the nine-year period of this study, it is important to note that this is likely an inadequate length of time to evaluate long-term population level changes (Pollard 1988); there was a large gap in survey years, and the sampling methods used over the period were not absolutely consistent. Insect populations notoriously undergo large fluctuations (Medeiros et al. 2015). The wide variations in abundance found intra-seasonally in 2018 and 2019 support that tenet and provide a hint of how the inadequate and irregular sampling of *M. blackburni* has likely obscured long-term trends in abundance. For example, in both 2018 and 2019 there was a dramatic difference between the relative abundances observed in February and March compared to those observed in April. Relative abundance trends including only a single survey from 2018 and 2019 could either indicate highly significant declines, if data from a low abundance month such as February or March was used, or no change at all, if relative abundance was estimated using data from the high abundance month of April. The difference of one month would yield very different conclusions regarding trends in abundance. It is also impossible to determine if the surveys in 2011, 2012, and 2017 occurred during a high or low abundance period for the moth. Because *M. blackburni* is an endangered species, it is prudent to adopt a conservative approach when estimating abundance trends. Thus, multiple surveys per year are needed to capture natural fluctuations in abundance and accurately assess long term trends, and the extrinsic and intrinsic factors that impact them. Ongoing surveys would also enable the calculation of detection probability for this species following the methods outlined by Lindzey and Connor (2011). Once detection probability has been established, it would be possible to estimate population size from raw counts of immatures. Quantifying the population of *M. blackburni* would provide a more accurate metric for determining population status. Furthermore, monthly surveys throughout the entire year would clarify the true seasonality of *M. blackburni*, providing a useful tool for planning future research and management activities.

This study was also geographically limited and evaluated changes in only one region where the moth is known to occur. Given the disjunct distribution of *M. blackburni* and its status as an endangered species, it would be unwise to assume that observed changes in relative abundance for the population occurring in the North Kona district are representative of those on

other islands, without further study. Trends in abundance may vary widely based on habitat quality and predator distributions, as well as abiotic factors like local weather conditions. Randomized sampling across the entire range of the moth on each island would be more likely to capture a greater portion of that expected variation in abundance. However, extensive fluctuations in monthly abundance are also known from other native Hawaiian insects (Ramadan 2001, Medeiros et al. 2013) and are likely not island-specific for *M. blackburni*.

Despite limitations, this study indicates that M. blackburni populations need continued conservation efforts. Consequently, to more accurately evaluate the population status of this endangered species, multi-month surveys should be conducted on all three islands randomized throughout the moths known range. Also, there is a significant correlation between *N. glauca* abundance and both the presence and abundance of *M. blackburni.* It is therefore crucial that adequate host plant abundance is maintained to prevent continued declines of this iconic Hawaiian endemic sphinx moth.

Conservation of a declining species is contingent upon providing accurate and adequate data within the constraints of time, access, and available funding. Multi-month surveys of immatures demonstrated extreme intra-seasonal variations in relative abundance and indicated a general long-term decline in relative abundance. This study indicates that using survey data from immature stages that are easy to detect, count, and identify can provide the necessary information to quantify population status and identify long-term changes in population for species which cannot be monitored using more traditional methods. It also indicates that volunteers without formal training are willing to aid in conservation monitoring, even when surveying for the less charismatic immature life stages. In conclusion, monitoring immature abundance of species can provide the data necessary to evaluate population changes and help prevent the loss of declining species.

CHAPTER 2. OVIPOSITION PREFERENCES OF THE ENDANGERED BLACKBURN'S SPHINX MOTH, *MANDUCA BLACKBURNI* (LEPIDOPTERA: SPHINGIDAE), ON AN INVASIVE HOST PLANT

Introduction

The world is in the midst of a sixth great extinction event (Ceballos and Ehrlich 2003, Barnosky et al. 2011) which has recently been revealed to encompass a global decline in insect biodiversity in particular (Sanchez-Bao and Wyckhuys 2019, Brooks et al. 2012, Fonseca 2009, Conrad et al. 2006). Insects are the dominant terrestrial organisms on the planet and herbivorous insect species alone make up one-quarter of all macroscopic organisms. Thus plant-insect interactions are taxonomically diverse, geographically pervasive, and of broad economic and ecological importance (Bernays and Chapman 1994). Consequently, it is crucial to understand how phytophagous insects respond to the increasingly rapid pace of anthropogenically driven global environmental change.

Manduca blackburni (Butler 1880) is a federally listed endangered sphinx moth currently restricted to highly fragmented dry forest habitat on three of the seven main Hawaiian Islands (Riotte 1986). The moth is oligophagous and exclusively utilizes members of the Solanaceae as larval host plants (Zimmerman 1958, Riotte 1986, Rubinoff and San Jose 2010). The three species of the Hawaiian endemic genus *Nothocestrum* (NOTH) (Solanaceae) used by *M. blackburni* as their native larval host plant are now also federally listed endangered species (USFWS 2005). Subsequent reductions in larval host plant resources through habitat loss and fragmentation, competition from invasive species, and browsing by alien ungulates have pushed *M. blackburni* to the brink of extinction (Gagne and Howarth 1982). Given the important role of sphinx moths as pollinators in tropical communities, especially tropical dry forests (Haber and Frankie 1989, Amorim et al. 2009), the impacts of *M. blackburni's* rarity or disappearance may be far ranging; the decline of several endangered Hawaiian plants is thought to have resulted from the loss of their sphinx moth pollinator (USFWS 2007, Huppman 2013, Walsh et al. 2019). While there are concerted efforts by conservationists and land managers to restore Hawaian dry forests, which have seen more than a 90% reduction in their extent since the arrival of human settlers, many native plants, including NOTH, show poor seedling recruitment (Ammondt 2010,

Thaxton et al. 2012, Cabin 2013, Medeiros et al. 2014). An active and persistent pollinator community has been shown to improve seed production within Hawaiian dryland ecosystems (Aslan et al. 2018) and is an essential part of a long-term self-sustaining ecosystem (Handel 1997, Williams 2011). Conservation of *M. blackburni,* a large moth known to utilize multiple nectar sources from and with the potential to act as pollinator to multiple plant species, may be vital for successful dry forest restoration.

Despite the decline in NOTH populations, *M. blackburni* persists. This is likely due to the intentional horticultural introduction of tree tobacco, *Nicotiana glauca* Graham (NICO) (Solanaceae), to the Hawaiian Islands at the end of the 19th century. Native to northern Argentina (Godspeed 1954), NICO is listed in the Global Invasive Species Database (International Union for the Conservation of Nature 2019), and is considered a high-risk invasive species within the Hawaiian Islands (Institute of Pacific Islands Forestry 2018). Thriving in dry, disturbed habitats following introduction it proliferated throughout six of the seven main islands, establishing in coastal areas and those once covered in native dry forest (Bernice Pauahi Bishop Museum Botanical Collections Database 2019). This resulted in significant habitat overlap with *M. blackburni*, which underwent a rapid host range expansion onto the invasive weed. NICO quickly became known as the moth's common food plant, with larvae recorded from it as early as 1919 (Swezey 1924). In contrast to the dogma that invasive plants such as NICO are detrimental to native insects (Morales and Traveset 2009, Mattingly and Flory 2011, Litt et al. 2014, Sunny and Sharma 2015), within much of its current range, *M. blackburni* now primarily relies on this invasive larval host plant (Riotte 1986).

The implications of such a host range expansion are difficult to predict and can have rapid evolutionary repercussions (Singer et al. 1993, Kuussaari et al 2000, New 2013, Russo et al. 2014). Selection of a suitable oviposition site is crucial for phytophagous lepidopteran species whose larvae have limited vagility (Mayhew 1997, Kessler and Baldwin 2002, Potter 2010). As such, it is unsurprising that the factors which influence oviposition site choice are complex and can include numerous intrinsic and extrinsic factors such as host plant chemistry, suitability as a food plant, branching architecture, and conspecific competition (Marquis 1992, Mayhew 1997, Mira and Bernays 2002, Gibbs and Van Dyck 2009, Floater 2010, Mattingly and Flory 2011, Zhang et al. 2012). The Preference-Performance hypothesis postulates that an insect's ovipositional preferences should confer maximal fitness to their offspring (Jaenike 1978). With

numerous factors working in combination, they may affect selection in conflicting directions, making it impossible for the insects to find an oviposition site which optimizes all possible variables (Gripenberg et al. 2010), thereby requiring fitness trade-offs. Additionally, in the case of rapid host shift or expansion, the mechanisms driving such a linkage may not be able to keep up, leading to a decoupling of oviposition preference and offspring performance. This study was undertaken to identify the oviposition preferences of *M. blackburni* on NICO to facilitate targeted conservation of valuable reproductive resources and to highlight the rapid adaptation of a declining insect species in the face of anthropogenically driven biodiversity loss.

Methods

We observed naturally occuring populations of *M. blackburni* that oviposit on NICO at sites in Puʻu Waʻawaʻa and Puʻu Anahulu in the Kona district of western Hawaii Island within Hawaii County, Hawaii, for one week each month from February through April of 2018, coincident with the moth's peak active season (Riotte 1986). At each of a total of 60 randomly selected sites known to contain NICO, two 75m_2 belt transects were established. One transect was oriented parallel to the firebreaks used to access these remote sites. All possible points along this first transect which could create a transect perpendicular to the firebreak including NICO were distinguished, and one was randomly chosen. The second 25m x 3m transect was established at that point beginning immediately adjacent to the first transect, ensuring non-overlapping transects. All NICO with trunks originating within the transects were searched for the eggs and larvae of *M. blackburni*. We recorded the height above ground to the nearest quarter inch of all eggs found using a tape measure. Height measurements were later converted from imperial to metric measurement. The leaf surface (upper or lower) upon which the egg had been deposited was also noted. Every effort was made to search the plants exhaustively, regardless of height or leaf density, and the percentage of the plant which had been searched was estimated. The height of each NICO plant was visually estimated and plants were categorized into four height groups. Height group one included plants less than 1 m. Height group two contained plants 1 - 2 m tall. Height group three contained plants 2 - 5 m tall. Height group four comprised all plants greater than 5 m tall. Likewise, leaf density was estimated as the approximate percentage of branches with leaf coverage and grouped into low, medium, and high density. Low density was defined as <30% leaf coverage, medium density was 30%-60% leaf coverage, and

high density was >60% coverage (Fig. 2.1). The mean height (i.e., above ground level) of oviposition was estimated and the percentage of eggs laid on upper and lower leaf surfaces was compared. The oviposition preference for plant height and leaf density was tested using Pearson χ_2 tests. All analyses were conducted using JMP Pro v. 14.2 (SAS Institute).

Figure 2.1 Clockwise from left *Nicotiana glauca* with high leaf density, medium leaf density, and low leaf density.

Results

The mean oviposition height for *M. blackburni* eggs found (n = 321, sd = 0.512) across all sample dates was 1.62m. The lower surface of leaves received 88.47% (n = 284) of eggs while 10.90% (n=35) of eggs were laid on the upper surface of leaves. Only 0.62% (n = 2) of the eggs found were laid somewhere other than the upper or lower surface of a leaf (Fig. 2.2). Those eggs were laid on a leaf petiole and the exact margin of a leaf.

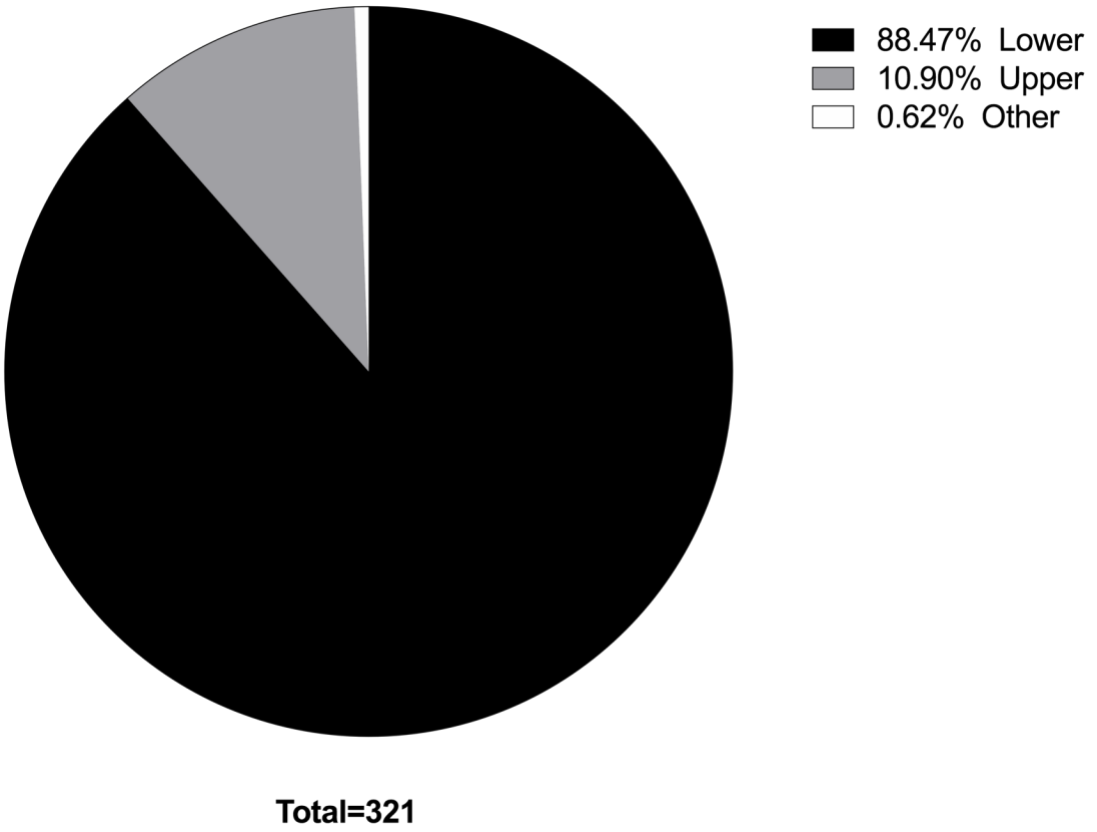

Figure 2.2 The percentages of eggs laid by leaf surface.

The contingency table analysis of oviposition by tree height indicates a statistically significant deviation from random ($\chi^2 = 46.05$, df = 3, $p < 0.0001$) (Table 2.1). Two-way contingency table analysis revealed oviposition preference for height group two was statistically significantly greater than height group one ($\chi^2 = 18.723$, df = 1, $p < 0.0001$). Preference for plants within height group three was higher than for both height group one ($\chi^2 = 40.744$, df = 1, $p < 0.0001$) and height group two ($\chi^2 = 10.66$, df = 1, $p = 0.0011$) but not significantly different from height group four ($\chi^2 = 0.165$, df = 1, $p = 0.6850$). Height group four was preferable for oviposition to height group one ($\chi^2 = 31.945$, df = 1, $p < 0.0001$), but was not statistically significantly different from height group two ($\chi^2 = 3.466$, df = 1, $p = 0.0626$) or height group three. There was a marked aversion to ovipositing on plants within height group one (< 1m tall). Plants within height group one made up 21% of the plants surveyed, yet they accounted for only 0.60% of eggs counted (Fig. 2.3).

Table 2.1. Frequency of oviposition by plant height group

Height Group	Plants	Plants with Eggs	% of Plants with Eggs	χ^2 value
0-1 m	168	1	0.60%	23.28*
1-2 m	225	30	13.33%	1.72
2-5 m	324	90	27.78%	12.83*
>5 m	69	17	24.64%	1.34
		$\chi^2 = 46.05$, df = 3, $p < 0.0001$		

* Indicates statistically significant deviation from random at $p < 0.005$.

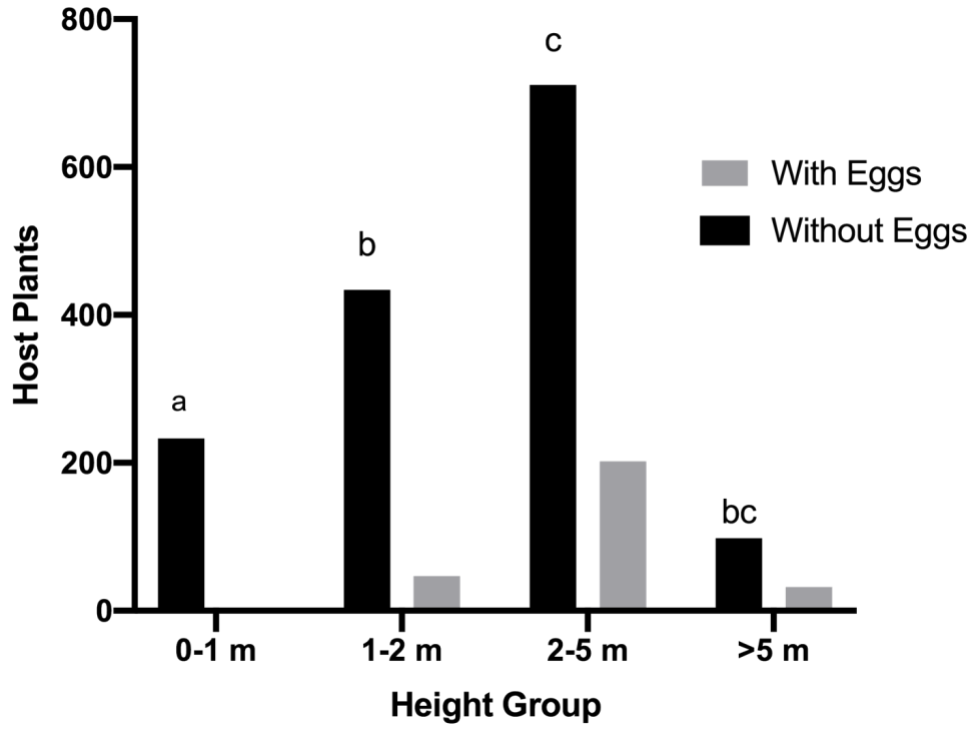

Fig.ure 2.3. The distribution of plants with and without eggs by height groups showing disproportionately high use of trees > 1 m tall for ovipositing. Columns not connected by the same letter are statistically significantly different at $\alpha \leq 0.05$.

Oviposition by leaf density also deviated from random ($\chi^2 = 41.61$, df = 2, p = <0.0001) (Table 2.2). Medium leaf density plants were prefered for oviposition over plants with low leaf density ($\chi^2 = 38.417$, df = 1, $p < 0.0001$) and high leaf density ($\chi^2 = 9.659$, df = 1, p = 0.0019). As all reachable branches were manually searched until completed, increases in leaf density

would not have affected our ability to find eggs. However, the distribution of leaf density was not random among height groups ($\chi^2 = 229.679$, df = 1, $p < 0.0001$,) which have a statistically significant impact on oviposition choice. Of the high leaf density plants, 63% were in height group one. To avoid potentially obfuscating density preferences, height group one plants were excluded and two-way contingency analyses were repeated. Among plants within height groups two, three, and four, there was a statistically significant preference for high leaf density plants over low leaf density plants ($\chi^2 = 21.071$, df = 1, $p < 0.0001$) and no difference between oviposition rates on medium and high leaf density plants ($\chi^2 = 0.324$, df =1 , $p = 0.5692$) (Fig. 2.4).

Table 2.2. Frequency of oviposition by plant leaf density

Leaf Density	Plants	Plants with Eggs	Percent of Plants with Eggs	χ^2 value
Low	395	30	7.59%	14.25*
Medium	394	93	23.6%	19.83*
High	135	15	11.11%	1.32
		$\chi^2 = 41.61$, df = 2, $p < 0.0001$		

* Indicates statistically significant deviation from random at $p < 0.001$.

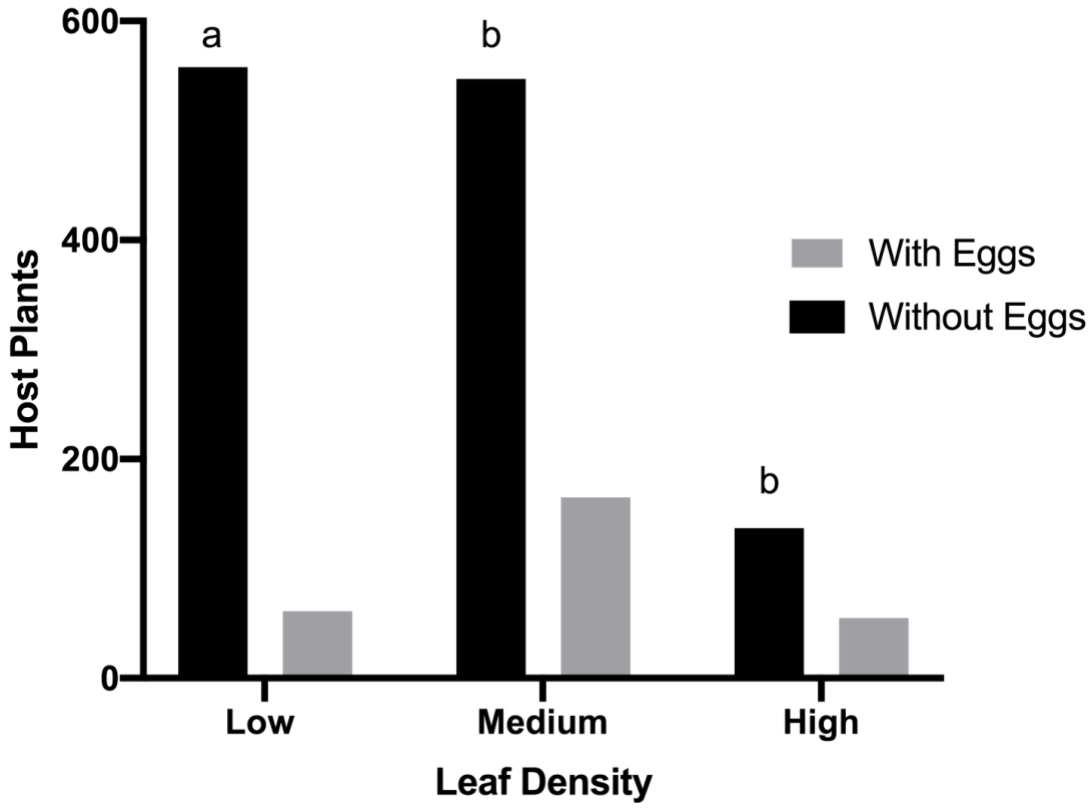

Figure 2.4 The distribution of plants with and without eggs by leaf density indicating the statistically significant disproportionate use of medium and high leaf density plants > than 1 m tall. Columns not connected by the same letter are statistically significantly different at $\alpha \leq 0.05$.

Discussion

In contrast to the closely related congeneric sphinx moth species *M. quinquemaculata* (Haworth) and *M. sexta* (Johans.) (Rubinoff et al. 2012, Kawahara et al. 2013), both important pests on solanaceous crops throughout the southern United States (Madden and Chamberlin 1945), little has been written about ovipositional behaviour of *M. blackburni.* As an endangered species once believed to be extinct, trenchant conservation efforts are needed to ensure the survival of this charismatic endemic moth. Yet without information on reproductive behavior, it is difficult to devise effective conservation strategies.

Whereas *M. quinquemaculata* and *M. sexta* typically utilize lower-growing herbaceous members of the Solanaceae as larval host plants, *M. blackburni*, pre-European contact relied on NOTH, which develops into soft-wooded trees ranging from 5-12 m tall, suggesting an adaptive shift (Gustafson et al. 2014). Since then, *M. blackburni* has been found on introduced

solanaceous plants typical of its congeners, and the larvae are able to develop on them to maturity (Zimmerman 1958, Riotte 1986, Rubinoff and San Jose 2010). NICO grows rapidly into a soft-wooded shrub and reaches reproductive maturity within months of germination (Schueller 2002). In the survey areas of this study, 45% of NICO plants surveyed were between 2-5 m tall and 9% of individuals had developed into trees greater than 5 m tall, some with trunks having a diameter at breast height exceeding 30 cm (personal observation). Thus, a mean oviposition height of 1.62 m fits well with the stature of the native host plants. This estimate may be lower than the true mean oviposition height as it was not always possible to completely search all leaves on taller plants due to the brittle nature of branches, the limitations of reach, and the necessity of avoiding damage to the larvae and eggs.

Like *M. quinquemaculata* and *M. sexta*, *M. blackburni* shows a strong preference for ovipositing on the underside of leaves (Madden and Chamberlin 1945, Bossart 1988). Oviposition on the underside of leaves is frequently noted for Lepidopterans and it is often attributed to leaf microclimates advantageous to the developing embryo (Williams 1981, Zelucki and Kitching 1982, Smits et al. 1986). Eggs laid on the underside of leaves might also experience reduced losses from heavy rainfall. In this study, 88.47% of eggs were laid on the lower surface of NICO leaves. However, despite a strong preference for ovipositing on the underside of leaves, *M. blackburni* eggs experienced high levels of mortality regardless of oviposition site (Chapter 3). The high rate of predator-driven mortality of *M. blackburni* eggs on NICO, coupled with the abundance of the novel host and the paucity of the historic host, seems to support the hypothesis of "bottom-up" selective forces governing this host range expansion rather than a "top-down" release from native host-specific predation or parasitism. Specifically, this host range expansion provided a salient increase in available reproductive and larval food resources, potentially rendering it adaptive even in the face of high egg mortality. Comparing larval performance, chemical defense, and resultant adult fecundity on native and non-native hosts would help to further elucidate the drivers of this host range expansion.

The data also show that *M. blackburni* prefer ovipositing on taller plants and virtually never lay eggs on plants less than one meter tall. Kessler and Baldwin (2002) found that *M. quinquemaculata* preferentially oviposit on the leaves found on the elongating portion of *Nicotiana attenuata* Torr. plants, rather than on the basal rosette of leaves. This seemingly puts the largely sessile developing larvae at a disadvantage in terms of food quality and availability,

newer leaves being smaller and shown to contain higher proportions of allelochemicals. However, it provides the advantage of reducing predation upon the eggs by *Geocoris pallens* Stal (Hemiptera: Geocoridae), an adventitious egg predator in the Hawaiian Islands since at least 1935 (Usinger 1936). In a field study in North America of a naturally occuring population of *M. quinquemaculata, G. pallens* accounted for all egg mortality with decreasing mortality levels as leaf height increased. Immature saplings of *Nothocestrum breviflorum* A.Gray, the only species found on Hawaii Island, produce leaves along the entire length of the developing trunk. However, in mature individuals, leaves are predominantly clustered at the tips of branches and would therefore not provide easy access for ground-dwelling predators. Increased oviposition height may have been selected for in *M. blackburni* to reduce egg predation and mortality from native ground dwelling predators, or as a recent adaptation to the advent of *G. pallens*. Interestingly, *M. blackburni* will oviposit on a variety of low-growing non-native solanaceous plants. Records indicate the moth historically used eggplant (*Solanum melongena)*, tomato (*Solanum lycopersicum)*, and tobacco (*Nicotiana tabacum)*, among others (Swezey 1924 Zimmerman 1958, Rubinoff and San Jose 2010). However, NICO has been considered the primary host for *M. blackburni* almost since its introduction (Swezey 1924). Preference for ovipositing on taller woody specimens of NICO could simply be reflective of shared characteristics with NOTH.

Furthermore, plants with higher leaf density provide a more attractive site for ovipostion over those plants with low leaf density. However, preference did not increase in proportion to leaf density as there was no statistically significant difference between oviposition on medium- and high-density plants after controlling for height. This suggests that the increase in oviposition on medium- and high-density plants is not strictly due to the increased availability of leaf surfaces. In Hawaii, NICO is a perennial plant which often grows rapidly following heavy rainfall, putting out a flush of numerous large deep green leaves. This form of growth was common in plants with high leaf density within this study. New leaves are less frequently used for oviposition despite the fact they provide a larger and potentially higher quality food source for developing larvae. This lack of preference for high leaf density plants may be due to higher concentrations of alkaloids found in the large-leafed new growth. Phylogenetic analyses place NOTH and NICO in different, distantly related subfamilies (Olmstead et al. 2008). While little is known about the phytochemistry of NOTH, the closely related and well-studied *Withania*

somnifera Dunal (Solanaceae) appears to be very different chemically from NICO (Dar et al. 2015). It is possible that the use of NOTH by *M. blackburni* has led to a reduced tolerance for the allelochemicals produced by NICO. Optimal Defense (OD) theory postulates that as leaves age, their value to the plant decreases (McKey 1974). Older, lower-value leaves are consequently allocated fewer defensive metabolites (Ohnmeiss and Baldwin 2000) . In keeping with OD, Kessler and Baldwin, 2002, found that nicotine concentration decreased with leaf age in *Nicotiana attenuate.* Deposition of eggs on the smaller, older leaves of NICO could limit the exposure of resultant larvae to toxic alkaloids. Further examination of the secondary chemistry of NOTH and the impact of dietary nicotine and anabasine levels on the development of *M. blackburni* larvae could either support or negate this theory and shed further light on the developmental needs and reproductive behaviour of this endangered moth.

NICO seems to provide a critical reproductive resource for *M. blackburni* in the highly modified landscape of Hawaiian dry forests, acting as an ecological replacement for the endangered *Nothocestrum* spp.. Consequently, despite its status as an invasive weed, preserving NICO as a conservation expedient is currently necessary given the scarcity of established populations of the native larval host plants and the difficulty of successfully outplanting NOTH (Medeiros et al. 2014) able to attain a size capable of supporting the voracious appetites of *M. blackburni* larvae. Therefore efforts should be made to preserve NICO plants which meet these preferred criteria during clearing projects. At the same time, given the invasive nature of NICO, as well as its ability to rapidly develop and form dense stands, removal is necessary at times, such as during habitat restoration or firebreak maintenance. This poses a significant challenge to land managers, as the removal of the invasive NICO reduces critical reproductive resources for endangered *M. blackburni*. The results of this study concur with the current best practice of removing NICO seedlings before they reach one meter tall. Given that less than 1% of eggs in this study were found on the smallest plants, their removal in order to maintain clear firebreaks would minimize negative impacts on existing *M. blackburni* populations. This type of conservation conundrum where a native species of conservation concern becomes reliant upon a non-native species is likely to become more common as biological invasions continue to increase and invasive species continue to become established in native ecosystems (Pearson and Callaway 2003). Studies evaluating the fitness trade-offs encountered during ecological replacement are needed to begin to unravel such riddles.

Notwithstanding the status of *M. blackburni* as an endangered species and its extensive use of NICO as a larval host plant, the impact of its host expansion on offspring performance and subsequent fecundity and oviposition choices has not been explicitly studied. No work has yet been done to evaluate and compare survivorship or performance of *M. blackburni* larvae on native and non-native larval host plants, though the moth historically fed on a wide-range of plants including tobacco, eggplant, tomato, and peppers. Likewise, the effect of natal diet on subsequent adult oviposition choices has not been investigated. Results from such research would not only be indispensable to any future captive rearing efforts but would also inform current conservation efforts which are crucial to the future of this iconic Hawaiian endemic.

CHAPTER 3. MORTALITY FACTORS OF THE ENDANGERED HAWAIIAN SPHINX MOTH, *MANDUCA BLACKBURNI* (SPHINGIDAE: LEPIDOPTERA) ON AN INVASIVE HOST PLANT, *NICOTIANA GLAUCA* (SOLANACEAE)

Introduction

The Hawaiian Archipelago is a biodiversity hotspot with high levels of endemism (Swezey 1929, Kaneshiro 1995), and more endangered species per unit area than anywhere else in the world (Mehrhoff 1993, Cabin 2013) including twenty-seven United States federally listed endangered insects (USFWS 2019). In light of the dramatic world-wide decline in insect biodiversity, there is a heightened need for targeted and effective conservation efforts (Thomas et al. 2004, Conrad et al. 2006, Fonseca 2009, Brooks et al. 2012, Sanchez-Bao and Wyckhuys 2019). Insect conservation is hampered by incomplete knowledge of their biology and ecology, including causes of mortality (New 2007, Krushelnycky et al. 2017). Ecological life tables are a cogent tool for evaluating sources of mortality in insects. While commonly applied to identify mortality factors that may control populations of pest species (Morris and Miller 1954;, Paradis and LaRoux 1965;, Harcourt 1969, Kuhar et al. 2002, Pustejovsky and Smith 2006;, Semeao et al. 2012, Pereira et al. 2018), ecological life tables can also confirm and quantify threats in the form of mortality factors, identify vulnerable life stages, and indicate suitable sites for reintroduction or population augmentation for native species. This can inform management practices and lead to improved recovery strategies for at-risk species.

The focus of this study is *Manduca blackburni* Butler (Lepidoptera: Sphingidae), a United States federally listed endangered sphinx moth endemic to the Hawaiian Islands. Adult flights and oviposition occur primarily after seasonal rains which initiate a flush of new growth in the larval host plant. In Hawaii, these rains typically occur over a period of several months between October and April. Eggs and larvae rarely found during the drier months of the year (Riotte 1986, Tessler 2015). Even during the active season, populations of immatures are low-density and the nocturnal adults are rarely seen. Eggs are smoothly ovoid to spherical, roughly 1.8 mm in diameter, and require approximately five days for the developing larva to hatch (Rubinoff and San Jose 2010). During that period, eggs progressively shift from bright translucent green to red-orange and ultimately to opaque beige on the day of hatching (personal observation).

M. blackburni larvae develop through five instars prior to burrowing into the ground to pupate. Estimated larval development time is approximately three weeks: two weeks to complete instars one through four, then an additional week as a fifth instar (Rubinoff and San Jose 2010). Like other closely related *Manduca* (Kessler and Baldwin 2002, Mira and Bernays 2002), *M. blackburni* larvae are believed to be relatively sessile, especially during the first four instars, and are thought to typically spend all five larval instars on their natal plant. Immediately prior to pupation, the fifth instar larva enters a wandering phase, leaving its natal host plant in search of a suitable spot to burrow, create its pupation chamber, and pupate.

Egg and larval development for *M. blackburni* are similar to other *Manduca* spp., such as *M. sexta* (Linnaeus) and *M. quinquemaculata* (Haworth), (Madden 1945, Bossart and Gage 1990). Unlike its congeners, which are economically important pests on solanaceous crops in the southeastern United States (Madden and Chamberlin 1945) and common throughout their widespread ranges (Holland 1968, Tuttle 2007, but see also Wagner 2012, Young et al 2017), *M. blackburni* populations have historically been exiguous (Swezey 1929, Rubinoff and San Jose 2010). Few specimens have been sighted since the 1940s, and the species was believed to be extinct in the 1970s after concerted search efforts failed to relocate it (Gagne and Howarth 1982, USFWS 2005). This scarcity can be partially explained by the destruction of Hawaiian dry forests and the precipitous population decline of the native larval host plants, three currently endangered species of the endemic genus *Nothocestrum* (Solanaceae) (USFW 1994, 2016). However, *M. blackburni* is oligophagous within the Solanaceae and this allowed the moth to utilize some nonnative plants, including *Nicotiana glauca* (Solanaceae). Native to northern Argentina, *N. glauca* was introduced as an ornamental to Hawaii in the late 1800s. *M. blackburni* has used the invasive weed as its primary larval host plant since at least the early 1900s (Swezey 1924, Riotte 1986). The current range of *N. glauca* includes six of the seven main Hawaiian Islands, where it is considered a high-risk invasive plant species by the USDA Forest Service PIER program (http://www.hear.org/pier/ accessed July 24, 2019.). Yet, *M. blackburni* populations only persist on three islands (USFWS 2005) at density levels well below what the invasive host plant could support (Rubinoff and San Jose 2010). This suggests factors other than host plant availability may be responsible for the suppression of *M. blackburni*.

Mortality from non-native parasitoid species has been postulated as a driving factor for both the prematurely declared extinction (Gagne and Howarth 1982, Riotte 1986) and the

current restricted range of *M. blackburni* (USFWS 2005, Rubinoff and San Jose 2010). This hypothesis may be supported by historically high rates of parasitism by the cosmopolitan egg parasitoid *Trichogramma* sp. in populations on Molokai, where the moth is no longer found (Williams 1947). In more recent times, Rubinoff and San Jose (2010) found no evidence of egg or larval parasitism in their small sample of six eggs and two larvae of *M. blackburni* from Maui. Likewise, studies evaluating the non-target effects of non-native parasitoids on endemic Hawaiian crambid moths (e.g., *Omiodes* spp. and *Udea stellate*) found that introduced parasitoids were likely not a primary cause in the declines of the focal native species (King et al. 2010, Kaufmann and Wright 2008).

I constructed partial ecological life tables for naturally occurring egg cohorts on the primary larval host plant *N. glauca* to quantify the impact of parasitoids and other mortality factors on *M. blackburni* populations and to estimate the species total mortality in the egg and larval stages. By identifying mortality sources and quantifying their relative contribution to species mortality, it is possible to determine major causes of ongoing rarity and target conservation efforts to the most urgent or tractable threats.

Methods

Survey Sites

I surveyed naturally occuring populations of *M. blackburni* on the invasive host plant *N. glauca*. Surveys were conducted each month from February through April of 2018 and 2019 at two locations in the Northern Kona district of western Hawaii Island within Hawaii County, Hawaii. I selected those dates to inlcude the moth's peak reproductive season (Riotte 1986). Puʻu Waʻawaʻa Cinder Cone State Park (hereafter Puʻu Waʻawaʻa) and Puʻu Anahulu Game Management Area (hereafter Puʻu Anahulu) were used as survey locations since biologists from the Hawaii Department of Land and Natural Resources (DLNR) had previously surveyed these areas for *M. blackburni* and mapped the distribution of its host plant. At Puʻu Waʻawaʻa, I randomly selected twenty-five locations known to be infested by the invasive host plant *N. glauca* to establish survey transects. A larger sampling effort was required at Puʻu Anahulu to achieve a statistically valid sample size due to the lower density of *M. blackburni* found there during previous surveys (Chapter 1). Therefore, I randomly selected thirty-five sampling points at Puʻu Anahulu. At each randomly selected point survey teams established two 75m^2 belt

transects. We oriented one 25 meter x 3 meter transect parallel to the firebreaks used to access the remote sites. Once the first transect was positioned, a second perpendicular 25 meter x 3 meter transect was placed at a random point adjacent to the first transect, ensuring non-overlapping transects, and including at least one host plant. At some sites, due to low host plant density, it was only possible to establish either a parallel or a perpendicular transect but not both.

Field Cohort Study

Survey teams searched all *N. glauca* with trunks originating within a transect. I recorded all eggs (N = 467) and any invertebrates found on surveyed plants. When establishing the egg cohorts, I classified eggs as: (1) predated if they were hollow, with a clear chorion and a hole too small to provide egress for a neonate larva; (2) parasitized if they were opaque pearlescent black (Flanders 1937, Kuhar et al. 2002); and (3) potentially viable if the eggs appeared developmentally normal. To facilitate the redetection of eggs or resulting larva during subsequent sampling, I tied flagging tape to branches with viable eggs and marked each leaf petiole bearing a viable egg with a permanent marker. I resurveyed plants with viable eggs after 12-14 days and continuing daily until all individuals had developed into fifth instar larvae or died. The approximately two-week survey interval minimized the possibility of larvae developing to the fifth instar, but dying or entering a wandering phase before they could be resurveyed. Upon resurvey, I classified egg mortality as: (1) predated if the egg was empty with a clear chorion and a small hole; (2) parasitized if the egg was an opaque pearlescent black; (3) disappeared if the egg could not be found and there was no sign of characteristic larval feeding damage, or if the leaf bearing the egg was no longer attached to the plant; (4) anthropogenically removed if the branch containing the egg was broken off of the plant and discarded; and (5) non-viable if the egg was still unhatched but not predated or parasitized. Larval mortality was classified as: (1) early larval mortality if the egg scar could be found and any feeding damage was minor and characteristic of the interveinal feeding habits of first, second, and early third instar *M. blackburni* but no larva could be found; (2) late larval mortality if there was copious feeding, leaving only leaf petioles, characteristic of the late third through fifth stadia, but no larva could be found; and (3) fifth instar if the surviving fifth instar larva was present. Parasitized eggs were collected and parasitoids reared for identification. Emerging parasitoids were macerated and slide mounted for identification using keys by Pinto (1998). Identifications were confirmed

by Dr. Mark Wright. In 2018, survey teams found 293 total eggs, including 149 viable eggs whose fates were monitored. In 2019, we found 174 total eggs including 62 viable eggs whose fates were monitored. *M. blackburni* individuals were only followed up to the fifth instar since this species pupates underground and, given their endangered status and the critical function of the pupal chamber in closely related *M. sexta* (Sprague and Woods 2015), further observation could have been fatal to the developing pupae.

Partial Life Table Construction

Because *M. blackburni* is a federally listed endangered species, naturally occurring egg cohorts were used out of necessity. As a consequence, the low-density populations, even during the known active season of the moth, often yielded cohorts with too few eggs for meaningful analysis (Fig. 3.1). For this same reason, I pooled eggs from both survey locations to create cohorts each month. A breakdown of the numbers entering each stage (l_x) and dying in each stage (d_x) by location can be found in Table 3.3. Additionally, with naturally occurring egg cohorts, the age of the egg when first surveyed varied within an approximately five-day range. During previous surveys (Chapter 1), research teams commonly found multiple developmental stages even on the same plant, indicating overlapping generations or a broad flight time for the species. It is possible that some eggs found already predated or parasitized would have been laid within the same five-day window as the viable eggs found, but some may equally well have persisted from previous oviposition events. I constructed two partial life tables to evaluate both the cohort which included all eggs found regardless of their initial condition (N = 467), and the cohort consisting of only viable eggs during the initial surveys (N = 211). These tables were constructed using the method described by Kaufman and Wright (2008) where l_x indicates the number of individuals entering each stage and d_x is the number dying in a given stage. For individuals which perished during the egg phase, I was able to determine d_xF as the mortality factor acting during that stage. Apparent mortality, or the proportion dying in a given stage, was indicated by q_x and was calculated by dividing d_x of a stage by its corresponding l_x value.

Marginal Attack Rates

In the study system, five distinct mortality factors (Tables 3.1 and 3.2) acted contemporaneously upon individuals within the egg stage. In order to compare the strength of

those mortality factors, I estimated marginal attack rates, m_x, defined as the level of mortality that would have occurred if that factor had been the only source of mortality (Royama 1981, Bellows et al. 1992, Elkinton et al. 1992). Given the limited knowledge on competition between mortality agents or temporal changes in their attack rates, I calculated marginal attack rates as:

$$m_i = 1 - (1 - q)^{q_i/q}$$

Where m_i is the marginal probability of attack from the ith cause, q_i is the apparent mortality from the ith cause, and q is the mortality rate from all factors combined (Elkinton et al. 1992).

Results

Across both data sets, disappearance and predation were the most significant mortality factors affecting the egg stage. Out of all surveys, I only observed two individuals which survived to fifth instar, yielding estimated mortality rates for both data sets above 99%.

For the data set consisting of all eggs found (Table 3.1), 89.3% (416/467), 9.2% (43/467), and 1.1% (5/467) died in egg, early instar, and late instar stages, respectively. Given the larger number of individuals included in this data set, only 0.4% (2/467) survived to fifth instar. Predation (68.5%) was the primary cause of egg mortality, followed by disappearance (27.9%). Inviability (1.4%), parasitism (1.2%) and anthropogenic causes (1.0%) made up the remaining causes of mortality within the egg stage. All parasitism was caused by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and resulted in the characteristic blackening of the host egg which persisted even after parasitoid emergence. Apparent mortality for the egg stage was 89.3%. Early instar larvae had an apparent mortality of 86.0% while late instar larvae had an apparent mortality of 71.4%. Specific mortality factors could not be determined for larvae.

Table 3.1 Partial life table for *M. blackburni* combining all cohorts by stage

Stage	*lx*	Mortality factor	*dx*	*qx*	*mx*
Egg	467	Predation	285	0.610	0.783
		Parasitism	5	0.011	0.026
		Disappearance	116	0.248	0.463
		Anthropogenic	4	0.009	0.021
		Non-viable	6	0.028	0.037
		Total	416	0.893	
Early instar	50		43	0.860	
Late instar	7		5	0.714	
			Total	Mortality=	99.572%

l_x Number entering each stage, d_x number dying during each stage, q_x proportion dying at each stage, m_x marginal attack rate

When considering only eggs identified as potentially viable during initial surveys (Table 3.2), 76.3% (161/211), 20.4% (43/211), and 2.4% (5/211) perished in egg, early instar, and late instar stages, respectively. Only 0.9% (2/211) survived to become fifth instars. Disappearance (72.0%) was the primary cause of egg mortality. Predation (16.6%) was the second largest cause of egg mortality. Inviability (2.8%) and anthropogenic causes (1.9%) were the remaining causes of mortality during the egg stage. Apparent mortality for eggs initially identified as potentially viable was 76.3%. Early and late instar stages had apparent mortality of 86.0% and 71.4% respectively. Apparent mortality rates for larvae did not vary between life tables as only viable eggs could develop into larvae.

Table 3.2. Partial life table for *M. blackburni* combining all viable cohorts by stage

Stage	*lx*	Mortality factor	*dx*	*qx*	*mx*
Egg	211	Predation	35	0.166	0.269
		Parasitism	0	0.000	0.000
		Disappearance	116	0.550	0.646
		Anthropogenic	4	0.019	0.035
		Non-viable	6	0.028	0.052
		Total	161	0.763	
Early larvae	50		43	0.860	
Late larvae	7		5	0.714	

Total Mortality = 99.052%

l_x Number entering each stage, d_x number dying during each stage, q_x proportion dying at each stage, m_x marginal attack rate

Table 3.3. Numbers of *M. blackburni* entering and dying in each stage by location

Stage	*lx* Pu‘u Anahulu	*lx* Pu‘u Wa‘awa‘a	Mortality Factor	*dx* Pu‘u Anahulu	*dx* Pu‘u Wa‘awa‘a
Egg	111	356	Predation	59	226
viable	57	154	Disappearance	25	91
predated	50	200	Parasitism	4	1
parasitized	4	1	Anthropogenic	4	0
non-viable	0	1	Non-Viable	4	3
Early Instar	15	35	Early Instar	14	29
Late Instar	1	6	Late Instar	1	4

l_x Number entering each stage, d_x number dying during each stage

Discussion

Both data sets are relatively congruent and estimate high levels of apparent mortality (q_x) during all developmental stages observed. This indicates that *M. blackburni* is vulnerable throughout its developmental period to a variety of mortality factors. I found high total mortality in both data sets, with less than 1% of eggs surviving to the final larval instar. Five of the six

cohorts exhibited 100% mortality, yet at least small numbers of eggs were detected during each month of surveys. It is possible that the estimated mortality may have been erroneously high if larvae left their natal host plant and travelled farther than the next closest plant. This would result in falsely assuming missing larvae had perished, thereby artificially inflating mortality. Another possibilty is that fecundity is high enough for the population to persist even at these estimated levels of mortality. While the number of eggs produced by *M. blackburni* has not been measured, wild-caught female individuals of the close congener, *M. sexta* lay an average 300 eggs (Madden and Chamberlin 1945) and the sister species, *M. quinquemaculata* (Rubinoff et al. 2012, Kawahara et al. 2013), averages 260 eggs per female (Svec 1964). If *M. blackburni* has similar laying capabilities, it would be able to maintain populations if total mortality to reproductive age was similar to the values estimated here (Wagner 2012). It is imprtant to note that these partial life tables only estimated mortality to fifth instar and additional mortality would be expected in the pupal and pre-reproductive stages. Mortality may also vary geographically, with some populations of this highly vagile moth experiencing lower rates outside of the survey area. Those subsequent generations may then expand to occupy the survey area. Broader distribution of survey sites would allow for the discovery of potential refugia and further clarify mortality rates and key mortality factors for *M. blackburni* at the landscape level. Measurement of female fecundity would also shed light on the reproductive capabilities for this species.

The primary discrepancy between the data sets is the relative impacts of two mortality factors acting on the egg stage: predation and egg disappearance. It is likely the persistence of predated eggs from previous laying events may have artificially inflated the number of predated eggs found during initial cohort surveys. Studies have shown that the age of insect eggs affects their attractiveness and vulnerability to arthropod predators and parasitoids (Colazza et al. 2004, Fatouros et al. 2012). Therefore, the higher number of predated eggs encountered during initial surveys may also, in part, be an artfact of the increased attractiveness of younger eggs. Within life table studies, disappearance itself is often attributed to predators (Miranda et al. 1998, Semeao et al. 2012). Given that predation and disappearance, as defined in this study, accounted for the majority of eggs lost in both data sets, it is clear that *M. blackburni* is highly vulnerable to egg predation.

The high mortality due to predation is consistent with other studies on congeneric species (Kessler and Baldwin 2002, Mira and Bernays 2002) which found predation to be the most

significant mortality factor during the egg stage. The predation described in both studies was also found on *M. blackburni* eggs, yielding empty eggs which appeared hollow with an intact chorion, barring a small hole near the apex of the egg. This type of predation on eggs of the sister species *M. quinquemaculata* is well known and ascribed to *Geocoris pallens* Stal, 1854 (Hemiptera: Geocoridae) (Kessler and Baldwin 2001, 2002; Schuman et al. 2013). *Geocoris pallens* is adventive on Hawaii Island (Usinger 1936, Zimmerman 1958), and a possible source for the predation found. However, other carnivorous insects known to predate lepidopteran eggs are present within the survey area, including *Orius persequens* (White, 1877) (Hemiptera: Anthocoridae). Mirids, juvenile reduviids, as well as larval coccinellids and chrysopids were also found on *N. glauca* within transects during surveys (personal observation), and could possibly produce the egg predation observed. To determine the identity of the predator, predaceous arthropods could be collected from within transects and offered eggs from a sphingid species of little conservation concern. Feeding damage could then be compared to predation observed in the field on *M. blackburni* eggs. Video monitoring of eggs could also prove fruitful, but is limited by the small number of eggs available and the expense of monitoring equipment. Identifying the predator or predators responsible for consuming eggs of *M. blackburni* and determining current distributions would allow for possible control efforts where the predators are established and identify potential refugia for future studies and population augmentation plans.

Egg disappearance, the other major contributor to egg mortality, could at least partially be attributed to the carnivorous insects encountered during surveys. In particular, three invasive ant species were frequently encountered throughout the survey areas: *Ochetellus glaber* (Mayr, 1862), *Pheidole megacephala* (Fabricius, 1793), and to a lesser extent, *Linepithema humile* (Mayr, 1868). Numerous individuals of at least one and often multiple species were found in every transect surveyed, actively tending high densities of honeydew producing insects on *N. glauca*. Though the ubiquity of these ants precluded direct analysis of their impact on *M. blackburni* mortality, they are likely major drivers of egg disappearance and pose a serious threat to *M. blackburni*. Previous studies have shown invasive ants to have a significant negative impact on native arthropod populations (Way et al. 1992, Gonring et al. 2003, Wetterer 2007, Lach et al. 2016, Krushelnycky 2017). Within the Hawaiian Islands, *P. megacephala* and *L. humile* and are considered two of the most harmful ant species (Loope and Krushelnycky 2007). Their advent and subsequent spread have had dramatic repercussions on native invertebrate

fauna. During future surveys, it would be possible to create additional transects whose ant populations could be supressed through the application of ant specific toxic baits. Mortality rates could then be compared with transects lacking such control methods to determine whether suppression of invasive ant populations increases the survivorship of *M. blackburni.*

Non-native parasitoids have been implicated in the decline of *M. blackburni* populations (Gagne and Howarth 1982, Riotte 1986, USFWS 2005, Rubinoff and San Jose 2010). Four species of *Trichogramma* are established on Hawaii Island, including the purposely introduced *T. japonicum* and the endemic *T. semifumatum.* Surprisingly, parasitism was rarely encountered during the two years of this study and only by *T. pretiosum*, believed to be adventive in origin (Nishida 2002). While it is impossible to determine the previous impact of parasitoids on historic populations of *M. blackburni,* these ecological life tables suggest that parasitoids no longer constrain current populations, at least within the survey areas. Long-term monitoring, including populations from additional islands, may be required to determine the broader impact of parasitism on *M. blackburni* populations.

In conclusion, such high levels of predation and mortality across all life stages of *M. blackburni* are of great conservation concern given the precarious status of this moth. However, in light of the temporal and geographic limitations of this study, it is important to regard these findings with caution. Long-term monitoring, including a broader geographic distribution, is needed to accurately understand the population dynamics of this resilient Hawaiian endemic. Such monitoring would have the added benefit of indicating regions best suited to future conservation plans such as captive breeding, population augmentation, or reintroduction. Confirmed identification of the predator or predators responsible for the distinctive egg predation observed and elucidation of the impacts of invasive ant species could be additional means of increasing recovery of the iconic *M. blackburni*.

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